LISTING OF CLAIMS

Claim 1 (Previously Presented): A method for detecting pathogenic mycobacteria in clinical specimens, said method comprising steps of:

(a) clarifying the clinical specimens from containment by conventional methods,

(b) treating the processed clinical specimens obtained in step (a) with a modified lysis buffer to inactivate live pathogenic mycobacteria to make the process safe for the user,

(c) extracting genomic DNA from the processed clinical specimen obtained from step (b) using a modified method to increase the yield and quality of DNA,

(d) designing sequence of SEQ ID No. 4 from the DNA obtained in step (c) for specific detection of pathogenic mycobacteria, said designed sequence comprising of selected intergenic region of SEQ ID. No. 3, a flanking region containing a portion of the gene mmaA1 of SEQ ID No. 1 and a portion of gene mmaA2 of SEQ ID No. 2 of the DNA obtained in step (c),

(e) designing and synthesizing a set of specific oligonucleotide primers of SEQ ID No. 5, which is the forward primer and SEQ ID No. 6, which is the reverse primer for Polymerase Chain Reaction (PCR) amplification of SEQ ID No. 4,

(f) developing a PCR amplification process for specific amplification of SEQ ID No. 4 of (d), said process comprising using the specific oligonucleotide primers designed and synthesized in step (e) for detecting presence of pathogenic mycobacteria in the clinical specimens, and

(g) analysing the amplified PCR product by restriction fragment length polymorphism (RFLP) analysis for differentiation of the species of the pathogenic mycobacterium for a quick assessment of HIV co-infection.

Claim 2 (Previously Presented): A method as claimed in claim 1, wherein the designed SEQ ID No. 4 has a sequence as follows:

5'TGGATCCGTTGACCATGAGGTGTAATGCCTTTCCGGACCCTAGGTGG
CCTTTCGGTGCTTGCACGGAACGCACCGATGCTTCCCCCTCCCCGCATG
CTCGAGGCATGCTATCCGATACAGGGCCGCCGCACTAAACCGCGATCG
AATTTGCCCAGGTCAGGGAACGGATATGAGCGGACGAGCTACTTGGTC
ATGGTGAACTGGGCGACGTTGATTAGGCCTCTGCGGAAGCGCTCCGCG
CATCCGGTCAGATAGTGCATGAAGTTGTTGTAGACCTCTTCGGACTGTA
CGGCGATGGCGCGTTCGCGGGCAGCCTGTAGGTTGGCGGCCCATGCAT
CGAGAGTCCGTGCGTAGTGGGAATTC 3'.

Claim 3 (Previously Presented): A method as claimed in claim 1, wherein the clinical specimen is selected from the group consisting of sputum, gastric lavage, cerebrospinal fluid, blood, tissue biopsies, bone marrow aspirates and other body fluids or tissues.

Claim 4 (Previously Presented): A method as claimed in claim 1, wherein clarification of the specimens in step (a) from the contaminants is carried out by adding to said specimens a digestion decontamination mix containing mild alkali, NaOH, tri sodium citrate and a mucolytic agent and guanidinium isothiocyanate in the range of about 0.4-2.5 M followed by concentrating the specimens from centrifugation.

Claim 5 (Previously Presented): A method as claimed in claim 4, wherein the digestion decontamination mix containing mild alkali, NaOH, tri sodium citrate and a mucolytic agent and guanidinium isothiocyanate in the range of about 0.5-2.0 M.

Claim 6 (Previously Presented): A method as claimed in claim 1, wherein the DNA in step (c) is extracted from the treated clinical specimen using a modified lysis buffer by inclusion of ingredients comprising guanidinium isothiocyanate in a range of about 0.5-8 M, Tris.Cl pH 7.6 in a range of about 20-100 mM, N lauryl Sarcosyl in a range of about 0.5-2% by weight of the buffer, EDTA in a range of about 0.1-20 mM, β-Mercaptoethanol in a range of about 1-25 mM and NaCl is present in an amount of about 0.2M; and purifying the DNA to improve yield by thorough precipitation by organic

solvents.

Claim 7 (Previously Presented) A method as claimed in claim 6, wherein guanidinium isothiocyanate is about 4M, Tris-HCl pH 7.6 is about 50 mM, N lauryl Sacrocsyl is about 1% by weight of the buffer, EDTA is about 1 mM, β-Mercaptoethanol is about 10 mM and NaCl is about 0.2M.

Claim 8 (Previously Presented): A method as claimed in claim 6, wherein the organic solvents are selected from the group consisting of a phenol/chloroform mixture and chloroform.

Claim 9 (Previously Presented): A method as claimed in claim 1, wherein the genomic DNA yield is increased in the range of about 25 to 50%.

Claim 10 (Previously Presented): A method as claimed in claim 9, wherein the genomic DNA yield is increased in the range of about 30 to 40%.

Claim 11 (Previously Presented): A method as claimed in claim 1, wherein high yielding amplification of DNA in step (f) is achieved by the modified Touch Down PCR cycling conditions, said conditions comprising steps of providing an initial high annealing temperature in the range of about 62-72°C followed by lowering of temperature in the range of about 0.1-1°C per PCR cycle for the first 10-25 cycles, then subsequently carrying out 30 PCR cycles at an optimum annealing temperature of about 56-62°C.

Claim 12 (Previously Presented): A method as claimed in claim 1, wherein high yielding amplification of DNA is achieved by modified Touch Down PCR cycling conditions, said conditions comprising steps of providing an initial high annealing temperature of about 70°C followed by lowering of temperature of about 0.8°C per PCR cycle for about first 14 cycles to about 58°C for another 25 PCR cycles.

Claim 13 (Previously Presented): A method as claimed in claim 1, wherein the

oligonucleotide primers capable of amplification of intergenic region of SEQ ID No. 4 for detection of pathogenic Mycobacteria in clinical specimens are selected from the group consisting of:

a. 5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID No. 5), which is the forward primer[[.]], and

b. 5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID No. 6), which is the reverse primer.

Claim 14 (Previously Presented): A method as claimed in claim 1, wherein the length of oligomeric primers is between 5 and 100 bases.

Claim 15 (Previously Presented): A method as claimed in claim 1, wherein the modified lysis buffer provides a cleaner preparation of the DNA.

Claim 16 (Previously Presented): A method as claimed in claim 1, wherein treatment with the modified lysis buffer containing 4M guanidinium isothiocyanate inactivates the live mycobacteria to make the procedure safer fro the operator.

Claim 17 (Previously Presented): A diagnostic kit for the detection of pathogenic mycobacteria in clinical specimens, comprising primers selected from the group consisting of:

a. 5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID No. 5), which is the forward primer, and

b. 5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID No. 6), which is the reverse primer.

Claim 18 (Previously Presented): A method as claimed in claim 1, wherein the contaminant clarified in step (a) comprises mucus and/or live organisms other than mycobacteria.

Claim 19 (New): A set of primers of SEQ ID No 5 and 6 comprising:

5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID No. 5), which is forward primer;

5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID No. 6), which is the reverse primer.